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(71) Applicant
Carl Schleicher & Schuell GmbH & Co. KG (FR Germany), Grimsehlstr 23, D-3352 Einbeck, Federal Republic of Germany

(72) Inventors
Dr Nils Hese,
Dr Andreas Clad,
Manfred Weisweiler

(74) Agent and/or Address for Service
Forrester Ketley & Co.,
Forrester House, 52 Bounds Green Road, London N11 2EY

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C7B

(54) **Electro-elution**

(57) A process for the electro-elution of electrically charged macromolecules by transferring the macromolecules from an elution space 1 into a trap under the action of an external electric field, wherein the arrangement is such that the macromolecules migrating under the influence of electric field first pass into a trap 3 through a membrane M2 which, in the electric field, is permeable to the macromolecules but, in the absence of the external electric field, is substantially impermeable to any macromolecules, and which does not permit transfer of the fluid medium in which the elution is being effected.

The trap may extend between the membrane M2 and a second membrane M1, the second membrane being permeable in the electric field to small ions, but not allowing any macromolecules to pass through, even in an electric field.

The process allows electro-elution from electrophoresis gels, for concentrating and for salinating biological molecules eg. DNA, RNA and proteins, with minimal loss of macromolecular material.

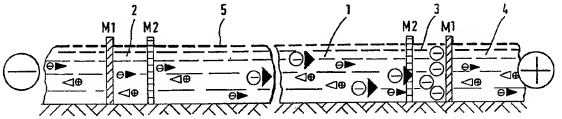

FIG.1

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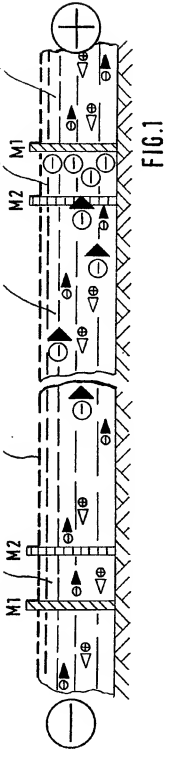

FIG.1

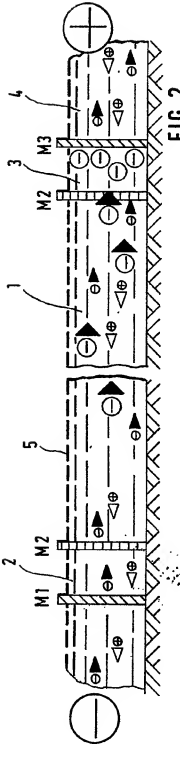

FIG.2

SPECIFICATION

Improvement in or relating to electro-elution

This invention relates to a process for the electro-elution of electrically charged macromolecules, and to an apparatus for carrying out the process.

In this specification electro-elution is to be understood not only as the elution of macromolecules from a gel, in particular an electrophoresis gel (that is to say the classical electro-elution) but also the elution of macromolecules from dilute solutions for the purpose of concentrating the solution of macromolecules. Electro-elution is also to be understood to include a process involving the transfer of macromolecules from a solution for purposes of desalination or for transferring appropriate molecular species into different buffer solutions (dialysis). The broad term "electro- elution" will be used to cover all these slightly different processes in the present specification.

In the present specification electrically charged macromolecules are considered to be electrically charged macromolecules having a molecular weight greater than one thousand. Examples of such macromolecules are biological macromolecules, such as in particular DNA, RNA or proteins.

The journal Analytical Biochemistry *124*, 299 to 302 (1982) discloses the electro-elution of electrically charged macromolecules from an elution space into a trap under the action of an external electric field. The macromolecules are eluted from an electrophoresis gel. In the described arrangement, the trap which is used to catch the eluted macromolecules is a small nylon bag which contains an adsorption gel, in particular malachite green gel, in which the macromolecules eluted from the electrophoresis gel are temporarily adsorbed. After the electro-elution process has been completed, the temporary adsorption gel is placed in a vertical column, corresponding to an ion-exhange column, and the macromolecules are eluted in a conventional way, using, in the example described, a one-molar sodium perchlorate solution. The losses of macromolecules caused by this temporary adsorption and subsequent conventional elution are at least about 25%. In other words a maximum yield of, at the most, 75%, can be obtained using this prior described process, which is among the best of the processes presently available. Moreover, the prior described process is very time consuming and expensive. It is especially expensive in view of the high material costs of, above all, the temporary adsorption gel. Malachite green gel costs, typically, in excess of £90 for 25 ml of the gel. Additionally the prior described process is time-consuming due to the necessity of performing the second elution step. Thus, the concentration of an electro-phoreses gel fraction by the known process requires a time of at least forty minutes.

According to one aspect of the present invention there is provided a process for the electro-elution of electrically charged macromolecules by transferring the macromolecules from an elution space into a trap under the action of an external electric field, wherein the arrangement is such that the macromolecules migrating under the influence of the electric field first pass into the trap through a first membrane, which, in the electric field, is permeable to the macromolecules but, in the absence of the external electric field, is substantially impermeable to any macromolecules, and which does not permit transfer of the fluid medium in which the elution is being effected.

Preferably the process is such that after passing through the first membrane the macromolecules are than passed towards a second membrane which is spaced from the first membrane, the trap being defined between the membranes, the second membrane being such that though permeable in the electric field to small ions of molecules, it does not allow any macromolecules to pass therethrough, even in an electric field. The macromolecules may subsequently be removed, together with a liquid medium, from the said trap.

In one embodiment two traps are provided, any negatively charged macromolecules being transferred, under the action of the electric field to one trap, and any positively charged macromolecules being transferred under the action of the electric field to the other trap.

Preferably, after the macromolecules have been transferred from the elution space into the trap or into the traps, the polarity of the electric field is briefly reversed, in order to detach any macromolecules which have collected on the said second membrane or second membranes which are impermeable to the macromolecules, so that the macromolecules are removed from the respective membrane and are transferred into the medium present in the trap.

It will be appreciated that the central concept of the invention comprises collecting the macromolecules, which have been eluted from the elution space under the action of an external electric field, in a trap which is preferably formed between two membranes. The membranes may be formed of appropriate polymer materials, and the trap may be initially be filled with distilled water or a buffer solution. This is, of course, very different from collecting the macromolecules in an adsorption trap, as has been proposed previously.

In a preferred process in accordance with the invention the eluted macromolecules are first passed through a first or inner trap membrane which, in the presence of the applied external electric field, is permeable even to macromolecules. The macromolecules are then passed on towards a second or outer trap membrane, but this second or outer trap membrane is impermeable to the macromolecules, even in the electric field. Thus the macromolecules are retained within the trap. It is preferred that the second or outer membrane should be permeable to small ions and molecules since then, any small ions or molecules which are drawn into the trap under the influence of the electric field will pass through the outer trap membrane. Thus, at least in theory, the trap should contain a virtually pure solution of the macromolecules. This enables

the process to be used to separate macromolecues from an electriophoresis gel, and also to desalinate the solution of the macromolecules.

After the electric field has been switched off no further macromolecules pass through the membranes defining the trap, since the inner trap membrane is of such a nature that it is impermeable to any macromolecular mass transfer when the electric field is not being applied. The first or inner trap membrane is also of such a nature that it does not permit transfer of the fluid medium in which the elution is being effected. Thus, in a typical case, the first or inner trap membrane may be considered as being substantially waterproof.

The arrangement is such that the macromolecules concentrated within the trap, together with the liquid medium enclosed within the trap, can readily be removed from the trap in a simple manner, for example by using a pipette.

A process in accordance with the invention may be utilised to carry out the electro-elution of electrically charged macromolecules with a yield of 90% or more. In a typical case not more than five minutes are required for eluting a typical electrophoresis gel fraction.

It is to be appreciated that, when utilising the process in accordance with the invention, it is not necessary to provide a temporary adsorption gel, as was required in the prior art, and thus the operating costs are reduced.

A problem that might arise when utilising a process in accordance with the invention is that when the macromolecules are transferred into the trap, they may accummulate on the second or outer trap membrane. As a result, the macromolecules cannot be quickly removed quantitatively with the liquid medium from the trap. This problem can be overcome, however, by adopting the expedient briefly described above of reversing the polarity of the electric field briefly after the actual electro-elution has been completed so that any electrically charged macromolecules accummulated on the second or outer trap membrane are detached from the membrane and migrate back into the fluid medium present in the trap. It is preferred that the field of reversed polarity be applied for between 10 and 15 seconds.

The above-mentioned problem may also be overcome by utilising membranes which do not adsorb or absorb negatively charged particles in any trap located adjacent the positive pole of the applied field, or which do not adsorb or absorb positively charged particles in a trap located adjacent the negative pole of the applied electric field. If such membranes are used the macromolecules will tend to remain in solution within the trap.

Of course, it is possible to utilise the application of field of reversed polarity, even if membranes are used which are intended not to adsorb or absorb charged particles.

According to a second aspect of this invention there is provided an apparatus for carrying out a process in accordance with the first aspect, said apparatus comprising an elution space at least partially bounded by a first membrane defining part of a macromolecule trap, said first membrane being permeable to macromolecules migrating under the action of an electric field, and being substantially impermeable to macromolecules in the absence of an electric field, and not permitting transfer of the fluid medium in which the elution is to be effected, the apparatus further comprising means for applying an external field to transfer macromolecules from the elution space to the trap.

Preferably an outer membrane which is impermeable to said macromolecules in an electric field, defining the trap together with said first membrane. Advantageously the elution space may be bounded by two traps, one located adjacent the positive pole of the external electric field, and the other located adjacent the negative pole of the external electric field. If desired, only one of the two traps may be provided with a second or outer membrane.

It will be appreciated that whilst an apparatus as described above may be utilised to elute simultaneously positively charged macromolecules into one trap and negatively charge macromolecules into the other trap, in most cases only macromolecules of one polarity will be of interest in any specific situation.

An apparatus, as described above, will ordinarily be utilised with a relatively highly concentrated buffer or electrolyte adjacent the electrodes which constitute the poles of the applied electric field. Thus, whilst it would be possible to utilise an equipment which did not incorporate a second or outer membranes associated with each trap, the trap would always be directly connected to the relatively highly concentrated buffer or electrolyte solution which may prove to be undesirable. However, if the trap is isolated from the space containing the electrodes by means of at least one closing membrane which, in the absence of an applied electric field, is virtually impermeable to any mass transfer, the liquid medium used in the trap can be substantially more dilute than the buffer solution used in the chamber adjacent the electrode. With particular advantage the liquid used in the trap or traps may be distilled water, and distilled water may even be used in the elution chamber.

Preferably the volume of the or each trap is ten times to one hundred times smaller than the volume of the elution space. An advantage of this is that the macromolecules may be substantially concentrated simultaneously with being transferred into the trap.

Preferably the membranes used can be wetted by the elution medium used or a buffer solution used in the apparatus. Advantageously the or each first membrane is impermeable to bacteria, even in the presence of an electric field. Preferably at least one of the membranes used is an asymmetrical membrane, the dense or "active" surface of which faces the interior of the relevant trap. Conveniently the second membrane of the trap located at the negative pole at the external electric field does not adsorb or absorb any positive macromolecules, and the second membrane of the trap located at the positive pole of the external field does not ad-

sorb or absorb any negative macromolecules.

Membranes having the requisite properties are readily available commercially, a wide variety of appropriate membranes being available from various suppliers. The membranes may be based on cellulose, cellulose esters, polyamides, polyamides and polysulfones. Membranes based on cellulose and cellulose esters, particularly cellulose acetate, are particularly preferred. For the first or inner trap membrane, a membrane having a pore size in the range of from 0.05 to 0.2 μm is preferred, whilst preferably the outer membranes are of such a type that they do not allow molecules of a molecular weight greater than 1000 to pass therethrough, not even in the presence of a relatively strong external electrical field.

A member suitable for use as the inner membrane, based on regenerated cellulose and having a mean pore size of less than or substantially equal to 0.2 μm is obtainable, for example, from the applicant company under the typed description R.S.B. Membranes suitable for use as an outer trap membrane, and impermeable in an applied electric field to macromolecules of a molecular weight greater than about a thousand are offered for sale by the applicant under the typed descriptions R.A.B. and R.C.B. Details of a wide selection of comparable membranes are, however, also to be found in the catalogues of other membrane manufacturers.

It is to be understood that the invention is not restricted to the various dimensional limits given, by way of example, above, but when the invention is put into practice the layout and combination of membranes will be selected in accordance with the specific elution problem to be solved at that time.

It is to be understood that the membranes may be supported or unsupported, reinforced or unreinforced, and may be of symmetrical or asymmetrical structure. The details of the specific problem to be solved will have to be taken into account when the appropriate choice is made. It is believed that the selection of an appropriate membrane is within the knowledge of the appropriate skilled man. When assymetrical membranes are used, however, care must be taken, since the dense or "active" surface of such a asymmetrical polymer membranes should preferably face the interior of the trap.

A process in accordance with the invention, and an apparatus in accordance with the invention may be employed above all for the electro-elution from electrophoresis gels, for concentrating and for salinating biological macromolecules such as, for example, D.N.A., R.N.A. or proteins. A further important field of application is the concentration of positively charged macromolecules, in particular positively charged proteins, at an appropriate adjusted pH value as an end in itself and ultimate object, or in combination with a simultaneous purification and concentration of negatively charged macromolecules i.e. in particular a separation of positively and negatively charged biological macromolecules.

In order that the invention may be more readily understood, and so that further features thereof may be appreciated, the invention will now be described, by way of example, with reference to the accompanyings in which:

Figure 1 is a diagrammatic representation of a first embodiment of the invention, and

Figure 2 is a similar diagrammatic representation of a second embodiment of the invention.

Figure 1 represents diagrammatically an elution space 1 having a macromolecule trap 2 located adjacent a negative pole of a means which applies an external electric field. A second trap 3 is located adjacent the positive pole of the means that applies the external electric field. An electrolyte or buffer 4 is present in the chambers which contain the poles applying the electric field, and distilled water may be present within the two traps and within the elution space 1, although the elution space 1 may be contain an appropriate buffer solution. An elecrophoresis gel containing negatively charged macromolecules is present within the elution space 1. The level 5 of liquid within the apparatus is such that the liquid does not reach the top of the membranes, but a substantial proportion of each membrane is submerged so that molecular transfer can take place through a substantial part of the area of each membrane.

In the arrangement represented in Figure 1 both the traps 2, 3, are defined between respectively identical membranes. Thus each trap has an inner membrane M2 of one type and an outer membrane M1 of a second type. The membranes M2 are such that macromolecules may pass through the membranes under the influence of an applied electric field. However the membranes M1 are such that macromolecules may not pass through those membranes under the influence of an applied electric field, whereas small molecules or ions may pass through the membranes M1 under the influencce of the applied field.

After the electric field has been switched on, the negatively charged macromolecules present in the elution space 1, symbolized in Figure 1 by a large circle containing a "–" sign, migrate from the elution space 1 through the first or inner trap membrane M2 of the trap 3, towards the outer trap membrane M1 of the trap 3. The macromolecules cannot pass through the outer trap membrane M1, even in the presence of the applied electric field. However, the smaller ions and molecules, such as any buffer ions from the medium in the elution space 1, (denoted by "-" signs and "–" signs surrounded by small circles) can readily pass through the membrane M1 and enter into the buffer solution M4 of the electrophoresis chamber surrounding the positive pole of the means applying the electric field. Thus the solution within the trap can effectively be desalinated. The negatively charged macromolecules collect on the outer trap membrane M1 of the trap 3. When substantially all the negatively charged macromolecules present in the elution space 1 have been transferred through the membrane M2 into the trap 3, the polarity of the applied electric current is reversed for a brief period of between 10 and 15 seconds. During this period of time the positive pole is located to the left

of the arrangement as shown in Figure 1, and the negative pole is located on the right. This causes a negatively charged macromolecule collected on the outer membrane M1 to be detached from the membrane M1 and to be transferred into the interior of the trap 3. The macromolecules can then be recovered quantitatively from this trap, for example by utilising a pipett transfer the liquid medium contained in the trap 3 to an appropriate receptacle.

With the electric field switched off, both the membranes M1, M2 are virtually impermeable to any mass transfer, and above all do not permit the transfer of the fluid medium in which the electrophoresis is being carried out, i.e. they are waterproof. Thus the flow of water is prevented from the elution space and from the electrophoresis chamber into the trap 3 during the time that the trap 3 is being emptied.

If positively charged macromolecules are to be eluted a similar process is preferred, but the molecules will be concentrated in the trap 2 adjacent the negative pole.

The apparatus shown in Figure 1 may also be utilised to separate positively and negatively charged macromolecules. In such a case the electrophoresis gel present in the elution space 1 will contain both species of molecule to be separated. When the electric field is applied the negatively charged macromolecules will pass into the trap 3, whereas the positively charged macromolecules will pass into the trap 2.

It is to be understood that since the relatively small molecules or ions may pass through the outer membranes M1 at the end of the electrophoresis process there is a substantially pure solution of macromolecules of the desired species present within the relevant traps.

Figure 2 corresponds generally to Figure 1, but in the arrangement shown in Figure 2 the outer membranes M1 and M3 of the two traps are not the same, but have slightly different properties. The membrane M1 of the trap 2 is conditioned in such a way that it does not adsorb or absorb any positive particles, whereas the outer trap membrane M3 of the trap 3 is conditioned in such a way that it does not adsorb or absorb any negatively charged particles. Of course the conditioning may not be perfect and may serve only to reduce the tendency of the respective membrane to adsorb or absorb the charged particles of the appropriate polarity.

When membranes of this type is used it is acceptable to apply a reverse polarity field for only a very brief period of time at the end of the electroelution step to ensure that substantially all the electrically charged macromolecules present in the trap are removed from the outer membrane of the relevant trap and are transferred into the liquid medium present within the trap.

In other respects the arrangement illustrated in Figure 2 operates in the same way as explained above in connection with Figure 1.

It is preferred that the inner membranes utilised in the traps are waterproof and are also impermeable to bacteria. In such a way it is possible to obtain a macromolecule concentrate which is absolutely free from bacterial, especially if the traps are initially filled with distilled water. It is to be noted that it is not possible to obtain such bacteria-free elution utilising processes presently available. Of course, it is to be understood that utilising the prior art processes may lead to a considerable loss of macromolecules, in particular those of biological origin, due to bacterial attack subsequent to the elution process.

CLAIMS

1. A process for the electro-elution of electrically charged macro-molecules by transferring the macromolecules form an elution space into a trap under the action of an external electrical field, wherein the arrangement is such that the macromolecules migrating under the influence of electric field first pass into a trap through a first membrane which, in the electric field, is permeable to the macromolecules but, in the absence of the external electric field, is substantially impermeable to any macromolecules, and which does not permit transfer of the fluid medium in which the elution is being effected.

2. A process according to claim 1 wherein after passing through the first membrane the macromolecules are then passed towards a second trap membrane which is spaced from the first membrane, the trap being defined between the membranes, the second membrane, the second membrane being such that though permeable in the electric field to small ions of molecules, it does not allow any macromolecules to pass therethrough, even in an electric field.

3. A process according to claim 1 or claim 2 wherein the macromolecules are subsequently removed, together with a liquid medium, from the said trap.

4. A process according to any one of the preceding claims wherein two traps are provided, any negatively charged macromolecules being transferred, under the action of electric field to one trap, and any positively charged macromolecules being transferred under the action of electric field to the other trap.

5. A process according to claim 2 or any claim dependent thereon wherein, after the macromolecules have been transferred from the elution space into the trap or traps, the polarity of electric field is briefly reversed, in order to detach any macromolecules which have collected on the membranes impermeable to the macromoleucles, so that the macromolecules are removed from the respective membrane or membranes and are transferred into the medium present in the respective trap or traps.

6. An apparatus for carrying out a process as claimed in any one of the preceding claims, comprising an elution space at least partially bounded by a first membrane defining part of a macromolecule trap, said first membrane being permeable to macromolecules migrating under the action of an external electric field, and being substantially im-

permeable to macromolecules in the absence of an external electric field, and not permitting transfer of the fluid medium in which the elution if to be effected, the apparatus further comprising means for applying an external electric field to transfer macromolecules from said elution space to said trap.

7. An apparatus as claimed in claim 6 wherein an outer membrane is provided which is impermeable to said macromolecules even in applied electric field, said outer membrane defining the trap together with the first membrane.

8. An apparatus as claimed in claim 6 or 7 wherein the elution space is bounded by two traps, one located adjacent the positive pole of the external electric field, the other located adjacent the negative pole of the external electric field.

9. An apparatus as claimed in any of claims 6 to 8 wherein the volume of the or each trap is ten times to one hundred times smaller than the volume of the elution space.

10. An apparatus as claimed in any one of claims 6 to 9 wherein the membranes can be wetted by the elution medium used or a buffer solution used in the apparatus.

11. An apparatus as claimed in any one of claims 6 to 10 wherein the or each first membrane is impermeable to bacteria, even in the presence of an electric field.

12. An apparatus as claimed in any one of claims 6 to 11 wherein at least one of the membranes is an asymmetrical membrane, the dense or "active" surface of which faces the interior the trap.

13. An apparatus as claimed in any one of claims 6 to 12 wherein the second membrane of the trap located at the negative pole of the external electric field does not significantly adsorb or absorb any positive macromolecules, and wherein the second membrane of the trap located at the positive pole of the external electric field does not significantly adsorb or absorb any negative macromolecules.

14. A process for the electro-elution of electrically charged macromolecules substantially as herein described with reference to Figure 1 of the accompanying drawings.

15. A process for the electro-elution of electrically charged macromolecules substantially as herein described with reference to Figure 2 of the accompanying drawings.

16. Macromolecules wherever eluted by a process according to any one of claims to or claims and

17. An apparatus substantially as herein described with reference to and as shown in Figure 1 of the accompanying drawings.

18. An apparatus substantially as herein described with reference to and as shown in Figure 2 of the accompanying drawings.

19. Any novel feature or combination of features disclosed herein.

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